Amendments to the Specification

Please delete the paragraph beginning on page 1, lines 20-29, and replace with the following amended paragraph:

Chemical tests are most widely used for FOBT. These tests typically require stool to be applied to paper impregnated with the chemical reagent guaiac. When developer solution is added to the paper, a blue colour develops with a positive result. Guaiac tests have the advantage of being inexpensive and easy to perform, but are less accurate (not specific for human blood) and less sensitive than desirable. Nevertheless, several international studies have shown that screening patients with these tests can save lives through the early detection of pre-cancerous and cancerous lesions. The commonly used guaiac tests detect the ~~haem~~heme of ~~haemo~~hemoglobin, and as this is relatively resistant to breakdown in the small intestine, these tests may detect bleeding anywhere within the intestinal tract. For colorectal cancer screening this is a disadvantage as these tumours are confined to the large intestine.

Please delete the paragraph beginning on page 2, lines 1-8, and replace with the following amended paragraph:

Recently more sensitive and specific immunological tests (e.g. immunochromatographic tests) have been developed that have the potential to improve the accuracy of detecting blood in screening for colorectal cancer. These tests typically detect the globin protein of ~~haemo~~hemoglobin, a protein that does not survive passage through the upper gastrointestinal tract. A positive immunological test therefore indicates lower gastrointestinal bleeding. In common with all immunologically based tests, however, these tests are subject to a "prozone" or "high dose hook" effect, where at high levels of analyte, the test may be inhibited to the extent that heavy bleeding may be missed.

Please delete the paragraph beginning on page 2, lines 10-20, and replace with the following amended paragraph:

Accordingly, there is a need to develop improved methods of detecting blood in biological samples which methods minimise the incidence of false negative results obtained due to the effects of the prozone phenomenon. In work leading up to the present invention, the inventor has developed a method of screening biological samples for the presence of blood utilising a two part testing procedure which comprises an immunological screen for the presence of the globin component of ~~haemo~~hemoglobin performed and a non-immunological screen for the ~~haem~~ heme component of ~~haemo~~hemoglobin. Accordingly, even if the immunological detection method utilised to screen for globin produces a false negative result due to the presence of high concentrations of globin, the ~~haem~~ heme test which is not sensitive to the effects of the prozone phenomenon will nevertheless produce a positive result. The inventors have also developed an immunological screening method which overcomes the effects of the prozone phenomenon.

Please delete the paragraph beginning on page 3, lines 9-11, and replace with the following amended paragraph:

(iii) permitting flowing of said biological sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 3, lines 24-26, and replace with the following amended paragraph:

(iii) permitting flowing of said gastrointestinal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 4, lines 9-11, and replace with the following amended paragraph:

(iii) permitting flowing of said gastrointestinal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 4, lines 24-26, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 4, lines 28-29, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a positive globin result is indicative of lower gastrointestinal tract bleeding.

Please delete the paragraph beginning on page 5, lines 12-14, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 5, lines 16-17, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a negative globin result is indicative of upper gastrointestinal tract bleeding.

Please delete the paragraph beginning on page 6, lines 1-3, and replace with the following amended paragraph:

(iii) permitting flowing of said biological sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 6, lines 16-18, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 6, line 20, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a positive globin result is indicative of colorectal cancer.

Please delete the paragraph beginning on page 9, lines 21-25, and replace with the following amended paragraph:

(ii) permitting flowing of said biological sample to a second region of said test matrix wherein said sample is placed in contact with an anti~~haem~~heme~~o~~globin antibody for a time and under conditions for a ~~haemo~~hemoglobin-anti~~haemo~~hemoglobin complex to form, immobilising said complex and detecting said complex by contacting said ~~haemo~~hemoglobin with an anti~~haemo~~hemoglobin conjugate; and

Please delete the paragraph beginning on page 9, lines 27-31, and replace with the following amended paragraph:

(iii) permitting flowing of uncomplexed conjugate to a third region of said test matrix wherein said uncomplexed conjugate is placed in contact with said ~~haemo~~hemoglobin, which ~~haemo~~hemoglobin is immobilised in said third region, for a time and under conditions sufficient for a ~~haemo~~hemoglobin-conjugate complex to form and detecting said complex;

Please delete the paragraph beginning on page 11, lines 3-12, and replace with the following amended paragraph:

The present invention is predicated, in part, on the development of a blood screening method which screens for both the globin component of ~~haemo~~hemoglobin and the ~~haem~~heme component of ~~haemo~~hemoglobin. By combining an immunological test for globin with a non-immunological test for ~~haem~~heme, the incidence of false negative results occurring due to the prozone phenomenon are minimised. The use of a two step testing procedure directed to testing for both the ~~haem~~heme and the globin components of ~~haemo~~hemoglobin also permits differentiation of upper gastrointestinal tract bleeding from lower gastrointestinal tract bleeding. There has also been developed an immunological based screening method which overcomes the analytically misleading test results which can be obtained when the prozone phenomenon occurs due to high analyte concentrations.

Please delete the paragraph beginning on page 11, lines 25-27 and replace with the following amended paragraph:

(iii) permitting flowing of said biological sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 12, lines 15-17, and replace with the following amended paragraph;

(iii) permitting flowing of said biological sample to a third region of said test matrix wherein said sample is placed in contact with guaiac or functional equivalent thereof for a time and under conditions sufficient for said guaiac to detect ~~haem~~heme.

Please delete the paragraph beginning on page 13, lines 22-24, and replace with the following amended paragraph:

(iii) permitting flowing of said gastrointestinal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 13, line 29 and ending on page 14, line 13, and replace with the following amended paragraph:

Reference to "test matrix" is a reference to any device which is suitable for sequentially testing a biological sample for the presence of the globin component of ~~haemo~~hemoglobin, utilising a immunological test, and the ~~haem~~heme component of ~~haemo~~hemoglobin, using a chromogen or functional equivalent thereof. In a particularly preferred embodiment, said test matrix is a chromatographic test strip which comprises a first region for receiving a biological sample and a second region which comprises two sections. The first section of the second region is an area of immobilised antiglobin antibody coupled to colloidal gold particles which are re-suspendible by a passing liquid front while the second section of the second region is an area of immobilised antiglobin capture antibody. The third region comprises an absorbent pad impregnated with guaiac. Alternatively, the third region may comprise a strip of guaiac-impregnated paper which is laminated to the second region. It should be understood that the three regions detailed in the present invention may be positioned sequentially or in some other manner, such as superimposed. For example, the first and second regions may be combined such that the sample is deliverable directly into the second region. The test matrix of the present invention may also comprise additional regions. For example, the present invention envisages the use of chromatographic strips which comprises an absorbent pad located after the third region.

Please delete the paragraph beginning on page 14, lines 15-29, and replace with the following amended paragraph:

Without limiting the present invention to any one theory or mode of action, according to a preferred aspect of the invention the biological sample which is applied to the first region wicks from the first region to the second region and the detection of globin and ~~haem~~heme is then performed as a sequential two step procedure. At the second region, the globin component of any ~~haemo~~hemoglobin which is present in the sample is bound by the antiglobin antibody coupled to the colloidal gold particles. The passing biological sample front re-suspends these antibodies and both the globin-antiglobin complex and the free anti-globin antibody wick from the first section of the second region to the second section. At the second section the globlin component of any ~~haemo~~hemoglobin present in the sample becomes bound to the immobilised antiglobin capture antibody while free antiglobin coupled to colloidal gold, the non-globin components of the biological sample and any excess globin which is not bound by the anti-globin antibodies of the second region continued to wick into the third region. At the third region, the ~~haem~~heme component of any ~~haemo~~hemoglobin which has not been captured at the second region reacts with a developer solution to cause the release of oxygen, which oxygen reacts with a chromogen such as guaiac to result in a colour change.

Please delete the paragraph beginning on page 15, lines 1-9, and replace with the following amended paragraph:

In the event that a biological sample comprises high concentrations of blood, and therefore high concentrations of ~~haemo~~hemoglobin, a false negative result may be obtained at the second region of the test matrix due to the prozone phenomenon. In this event, the third region of the test matrix, which detects the ~~haem~~heme component of ~~haemo~~hemoglobin based on the non-immunological chromogen reaction, will nevertheless produce a positive result. Accordingly, the incorporation of a non-immunological chromogen test with the immunological globin test provides a safe guard against obtaining false negative results due to the effects of the prozone phenomenon where high concentrations of blood are present in the sample.

Please delete the paragraph beginning on page 15, lines 11-19, and replace with the following amended paragraph:

The method of the present invention requires that the ~~haemo~~hemoglobin of the red blood cells is exposed prior to commencement of the test. This may be achieved by any one of a number of methods known to those skilled in the art. For example, contacting the biological sample with a red blood cell lysis solution, prior to commencement of the test, would achieve this object. It is also within the scope of this invention to cleave the ~~haem~~heme and the globin components of the ~~haemo~~hemoglobin either before the test begins or at some point before the biological sample wicks into the third region. In this way, it would be possible to minimize the incidence of the ~~haem~~heme component being trapped by the antiglobin capture antibodies by virtue of its attachment to the globin component.

Please delete the paragraph beginning on page 16, lines 9-11, and replace with the following amended paragraph:

(iii) permitting flowing of said gastrointestinal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 17, line 22 and ending on page 18, line 9, and replace with the following amended paragraph:

"Detecting" the formation of a globin-antiglobin complex or the chemical reaction between ~~haem~~heme and the chromogen may be by any convenient method which will be known to those skilled in the art. In the method of the invention exemplified herein, the antiglobin antibody which becomes resuspended by the wicking biological sample front is complexed with colloidal gold. As the globin-antiglobin/colloidal gold complex is trapped by the antiglobin capture antibody impregnated in the second section of the second region of the chromatography strip, the colloidal gold becomes visible as a pink band due to its increasing concentration during

trapping of the complex at this point. Alternatively, the antiglobin antibody may be radio-labelled or enzymatically labelled such that upon addition of a substrate a colour change is observed if globin is present. The detection of ~~haem~~heme by a chromogen is preferably achieved by the addition of a developer such as peroxide which reacts with ~~haem~~heme to produce water and oxygen. The oxygen which is liberated then reacts with the chromogen to produce a colour change. For example, when guaiac reacts with oxygen a blue colour is produced. The chromogen may be incorporated into the test matrix at the third region together with the developer or else the developer may be added as a liquid reagent at a later stage. If the developer is dried into the test matrix with the chromogen, then the paper will turn blue upon the arrival of aqueous ~~haemo~~hemoglobin. Alternatively, some other type of reporter molecule which detects the reactivity between the ~~haem~~heme and the chromogen or functional equivalent thereof may be used.

Please delete the paragraph beginning on page 18, lines 11-25, and replace with the following amended paragraph:

In a preferred aspect, the present invention is used to diagnose gastrointestinal tract bleeding by analysing faecal samples for the presence of blood. Without limiting the present invention to any one theory or mode of action, the chromogen test will positively identify bleeding from any part of the gastrointestinal tract (that is, both the upper and lower regions of the tract) since it detects the ~~haem~~heme component of ~~haemo~~hemoglobin and ~~haem~~heme is relatively resistant to breakdown in the small intestine (the upper gastrointestinal tract). The globin component of ~~haemo~~hemoglobin however, does not survive passage through the upper gastrointestinal tract. A positive globin result in a faecal sample therefore indicates that bleeding has occurred in the lower gastrointestinal tract. Accordingly, by applying a combined two step immunological and non-immunological based test, it is possible to differentiate between upper and lower gastrointestinal tract bleeding wherein a positive ~~haem~~heme result together with a negative globin result indicates upper gastrointestinal tract bleeding and a positive ~~haem~~heme result together with a positive globin result indicates lower gastrointestinal tract bleeding. This is of particular importance, for example to the diagnosis of colorectal cancer, the symptoms of which include lower gastrointestinal tract bleeding.

Please delete the paragraph beginning on page 19, lines 6-8, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 19, lines 10-11, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a positive globin result is indicative of lower gastrointestinal tract bleeding.

Please delete the paragraph beginning on page 19, lines 26-28, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 19, lines 30-31, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a negative globin result is indicative of upper gastrointestinal tract bleeding. Preferably said chromogen is guaiac or functional equivalent thereof.

Please delete the paragraph beginning on page 20, lines 14-16, and replace with the following amended paragraph:

(iii) permitting flowing of said biological sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme.

Please delete the paragraph beginning on page 20, lines 29-31, and replace with the following amended paragraph:

(iii) permitting flowing of said faecal sample to a third region of said test matrix wherein said sample is placed in contact with a chromogen or functional equivalent thereof for a time and under conditions sufficient for said chromogen to detect ~~haem~~heme;

Please delete the paragraph beginning on page 21, line 1, and replace with the following amended paragraph:

wherein a positive ~~haem~~heme result and a positive globin result is indicative of colorectal cancer.

Please delete the paragraph beginning on page 24, lines 1-20, and replace with the following amended paragraph:

By comparing the relative intensities of the analyte test results of the second region and the conjugate test results of the third region, it is possible to determine, with greater certainty than has previously been available, whether the analyte of interest is present in the subject biological sample. Due to the fact that this technique facilitates the correct interpretation of whether or not the analyte results of region two have been subject to the occurrence of the prozone phenomenon, the results also provide a general indication of the concentration of analyte present in the sample. For example, where the analyte of interest is ~~haemo~~hemoglobin, a biological sample which does not contain any ~~haemo~~hemoglobin will produce a negative result in the second region and a strongly positive result in the third region where all the available conjugate will ultimately become complexed with the immobilised ~~haemo~~hemoglobin. Where low ~~haemo~~hemoglobin concentrations are present in a test sample, a weak positive result would

be expected in the second region while a strong signal would be detected in the third region since the low level of ~~haemo~~hemoglobin initially present in the sample would have complexed only a small proportion of the total conjugate available for testing. Where high ~~haemo~~hemoglobin concentrations are present, the prozone phenomenon will result in a weak signal being detected in the second region together with a weak signal present in the third region where severely depleted conjugate concentration would become immobilised. Where extremely high ~~haemo~~hemoglobin concentrations are present it would be expected that no signal is detected in either the second or third regions due to the lack of free unbound conjugate and the nevertheless excessive concentrations of unbound ~~haemo~~hemoglobin.

Please delete the paragraph beginning on page 27, lines 15-19, and replace with the following amended paragraph:

(ii) permitting flowing of said biological sample to a second region of said test matrix wherein said sample is placed in contact with an anti~~haemo~~hemoglobin antibody for a time and under conditions for a ~~haemo~~hemoglobin-anti~~haemo~~hemoglobin complex to form, immobilising said complex and detecting said complex by contacting said ~~haemo~~hemoglobin with an anti~~haemo~~hemoglobin conjugate; and

Please delete the paragraph beginning on page 27, lines 21-25, and replace with the following amended paragraph:

(iii) permitting flowing of uncomplexed conjugate to a third region of said test matrix wherein said uncomplexed conjugate is placed in contact with said ~~haemo~~hemoglobin, which ~~haemo~~hemoglobin is immobilised in said third region, for a time and under conditions sufficient for a ~~haemo~~hemoglobin-conjugate complex to form, and detecting said complex;

Please delete the paragraph beginning on page 30, lines 4-7, and replace with the following amended paragraph:

Blood was diluted 1:100, 1:1000 and 1:10,000 in immunological test buffer. This buffer caused lysis of the red blood cells, so that the ~~haemo~~hemoglobin was liberated into solution. The diluted blood samples were added to wells of a microtitre plate. Three negative control wells were included, each containing buffer alone.

Please delete, page 30, lines 20-23, and replace with the following amended section heading:

EXAMPLE 3
EXPERIMENTAL DEMONSTRATION OF THE USE OF A THIRD (ANTIGEN) LINE IN AN IMMUNOCHROMATOGRAPHIC ASSAY FOR HUMAN ~~HAEMO~~HEMOGLOBIN (Hb)